

## Description of Additional Supplementary Files

**Supplementary Data 1. *In silico* reconstruction of the extracellular vesicle pathway in *Ixodes scapularis* ticks.** *I. scapularis*, *Mus musculus*, and *Drosophila melanogaster* orthologs for proteins involved in the EV biogenesis were identified using the PSI-BLAST tool in NCBI. Human proteins were used as query.

**Supplementary Data 2. High-throughput proteomics analysis of cargo proteins present in extracellular vesicles originated from *I. scapularis* salivary glands.** Cargo of salivary gland EVs identified by MS/MS mass spectrometry were blasted against the genome of *Homo sapiens* to identify orthologs.

**Supplementary Data 3. MS/MS spectra of peptides identified as “1 unique/protein ID” in the proteomics analysis of *Ixodes scapularis*.** Peptides and proteins were exported from PEAKS X+ with “ $-\log p > 20$ ” scores for peptides and proteins; corresponding to  $p$ -values  $< 0.01$  for both peptides and proteins identification.

**Supplementary Data 4. Gene code and protein identification for FunRich analysis.** Gene codes from human orthologs to tick proteins identified in salivary gland vesicles were input into FunRich. Data represents the codes of proteins found within the vesiclepedia database.

**Supplementary Data 5. Ingenuity pathway analysis of the protein cargo originated from *I. scapularis* extracellular vesicles.** Proteins identified with MS/MS were input into the Ingenuity Pathway Analysis software to determine over-represented pathways.

**Supplementary Data 6. Primers used for RNAi silencing and qRT-PCR.** Primer type, sequence, and location within the target gene are listed.

**Supplementary Data 7. Resources and reagents available.** Sources, vendors, and dilutions (when applicable) used during these studies.

**Supplementary Movie 1. Nanoparticle tracking analysis of nanovesicles purified from *Amblyomma americanum* AAE2 cells.**

**Supplementary Movie 2. Nanoparticle tracking analysis of nanovesicles purified from *Ixodes scapularis* ISE6 cells.**

**Supplementary Movie 3. Nanoparticle tracking analysis of nanovesicles purified from *Dermacentor andersoni* DAE100 cells.**

**Supplementary Movie 4. Nanoparticle tracking analysis of nanovesicles purified from ISE6 cells transfected with the scrambled RNA against the *I. scapularis* gene *vamp33*.**

**Supplementary Movie 5. Nanoparticle tracking analysis of nanovesicles purified from ISE6 cells transfected with the small interfering RNA against the *I. scapularis* gene *vamp33*.**

**Supplementary Movie 6. Nanoparticle tracking analysis of nanovesicles purified from ISE6 cells transfected with the scrambled RNA against the *I. scapularis* gene *synaptobrevin 2*.**

**Supplementary Movie 7. Nanoparticle tracking analysis of nanovesicles purified from ISE6 cells transfected with the small interfering RNA against the *I. scapularis* gene *synaptobrevin 2*.**

**Supplementary Movie 8. Nanoparticle tracking analysis of nanovesicles purified from *I. scapularis* salivary glands.**

**Supplementary Movie 9. Live cell imaging of nanovesicles derived from *I. scapularis* ISE6 cells bound to murine bone-marrow derived macrophages.**

**Supplementary Movie 10. Nanoparticle tracking analysis of nanovesicles purified from *I. scapularis* ISE6 uninfected cells.**

**Supplementary Movie 11. Nanoparticle tracking analysis of nanovesicles purified from *I. scapularis* ISE6 cells infected with the intracellular bacterium *Anaplasma phagocytophilum*.**

**Supplementary Movie 12. Nanoparticle tracking analysis of nanovesicles purified from *I. scapularis* ISE6 cells stimulated with the extracellular bacterium *Borrelia burgdorferi*.**

**Supplementary Movie 13. Nanoparticle tracking analysis of nanovesicles purified from *I. scapularis* uninfected ISE6 cells.**

**Supplementary Movie 14. Nanoparticle tracking analysis of nanovesicles purified from *I. scapularis* ISE6 cells infected with the parasite *Babesia microti*.**

**Supplementary Movie 15. Nanoparticle tracking analysis of nanovesicles purified from *D. andersoni* uninfected DAE100 cells.**

**Supplementary Movie 16. Nanoparticle tracking analysis of nanovesicles purified from *D. andersoni* DAE100 cells infected with *Francisella tularensis*.**

**Supplementary Movie 17. Nanoparticle tracking analysis of nanovesicles purified from uninfected *Amblyomma americanum* AAE2 cells.**

**Supplementary Movie 18. Nanoparticle tracking analysis of nanovesicles purified from *Amblyomma americanum* AAE2 cells infected with *Ehrlichia chaffeensis*.**

**Supplementary Movie 19. Nanoparticle tracking analysis of nanovesicles purified from *Dermacentor andersoni* salivary glands.**